Brief Definitive Report

Fibrinolytic Response to Tumor Necrosis Factor in Healthy Subjects

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Summary

Tumor necrosis factor (TNF) may be involved in the disturbance of the procoagulant-fibrinolytic balance in septicemia, leading to microvascular thrombosis. To assess the dynamics of the fibrinolytic response to TNF in humans, we performed a crossover saline-controlled study in six healthy men, investigating the effects of a bolus intravenous injection of recombinant human TNF (50 $\mu g/m^2$) on the stimulation and inhibition of plasminogen activation as well as on plasmin activity and inhibition. TNF induced a brief fourfold increase in the overall plasma plasminogen activator (PA) activity peaking after 1 h ($p < 0.0001$), which was associated with rises in the antigenic levels of urokinase-type plasminogen activator ($p < 0.0001$) and tissue-type plasminogen activator ($p < 0.0001$). Plasminogen activator inhibitor type I antigen remained unchanged in the first hour, but showed a rapid eightfold increase thereafter ($p < 0.0001$), which coincided with the decrease in PA activity. Generation of plasmin activity in the first hour was signified by an 11-fold rise in D-dimer levels ($p < 0.0001$); inhibition of plasmin was reflected by a 36-fold rise in plasmin-α_2 antiplasmin complexes ($p < 0.0001$), as well as by a transient 16% decrease in α_2-antiplasmin activity ($p < 0.01$). In conclusion, TNF induced an early activation of the fibrinolytic system becoming maximal in 1 h, with a rapid inhibition thereafter. Earlier observations in the same subjects showed sustained coagulation activation for 6–12 h. The observed disbalance between the procoagulant and fibrinolytic mechanisms after TNF injection confirms the in vivo relevance of the effects of TNF on vascular endothelium in vitro and may explain the tendency towards microvascular thrombosis in septicemia.

Disseminated intravascular coagulation is a serious and frequent complication of septicemia. In septicemia, activation of the coagulation cascade is accompanied by a biphasic change in the fibrinolytic system involving initial stimulation and subsequent inhibition of plasminogen activation (1). The net result of these concomitant coagulative and fibrinolytic responses in septic patients is microvascular thrombosis.

In recent years, evidence has accumulated that the cytokine TNF is a crucial factor in the initiation of the septic syndrome (2). In addition, several in vitro studies indicate that TNF may be an important factor in the hemostatic disbalance observed in septicemia (3–6). Investigations in cancer patients infused with recombinant TNF revealed an activation of both the coagulation and fibrinolytic system (7–9). However, the absence of sequential measurements, the heterogeneity of the study populations, and the preexistent subclinical activation of both the coagulation and fibrinolytic system hampers proper evaluation of the balance between these opposite pathways.

We have recently shown in a crossover saline-controlled study in six healthy men that a single intravenous injection of recombinant human TNF induced activation of the coagulation system that persisted for >6 h, (10). In the present investigation, performed simultaneously with the coagulation study, we monitored the fibrinolytic changes after injection of TNF. We then compared the time course of the fibrinolytic response to TNF with its procoagulant effects to hypothesize about the mechanism of the frequently observed extensive deposition of thrombi in the microvasculature of multiple organs in patients with septicemia.

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Materials and Methods

Study Design. The study was approved by the institutional research and ethics committees and written informed consent was obtained from all volunteers. The present study was done simultaneously with an investigation on the coagulative effects of TNF of which the results have been published previously (10). Six healthy male volunteers (age 27–33 yr) participated in the study. Each subject was studied twice with an interval of at least 3 wk; once after, a bolus intravenous injection of recombinant human TNF of 50 $\mu g/m^2$ (Boehringer Ingelheim, Ingelheim am Rhein, Germany), and once after, an equivalent volume of isotonic saline was administered.

Sampling and Assays. Blood samples were obtained by separate venipunctures. Blood (9 vol) for the measurement of PA activity, urokinase type plasminogen activator (u-PA) antigen, t-PA antigen, plasminogen activity, D-dimer and α_2-antiplasmin activity were collected in plastic syringes containing 3.2% sodium citrate (1 vol). Blood for the determination of PAI-1 antigen was collected in plastic syringes loaded with the following anticoagulant: 270 mM EDTA, 1.9 mM Na_2CO_3, 282 nmol/liter prostaglandin E_1, and 30 mM theophyline. For the measurement of plasmin-α_2-antiplasmin (PAP) complexes, t-PA-PAI-1 complexes, and u-PA-PAI-1 complexes blood was collected in in siliconized Vacutainer tubes (Becton Dickinson & Co., Plymouth, England) to which EDTA (10 mM) and Polybrene (0.05 percent; wt/vol) were added.

PA activity was measured by an amidolytical assay (11). The results are expressed as a percentage of pre-infusion PA activity. u-PA antigen, t-PA antigen, PAI-1 antigen, and D-dimer were measured with ELISAs (12–14). t-PA-PAI-1 and u-PA-PAI-1 complexes were determined with specific RIAs. Plasminogen activity and α_2-antiplasmin activity were measured by automated amidolytic techniques (15). The results are expressed as percentages of normal. PAP complexes were determined by RIA (16).

Statistical Analysis. Values are given as means ± SEM. Differences in results between the TNF and saline experiments were tested by analysis of variance (ANOVA) and Newman-Keul's test for multiple comparison, as indicated.

Results

Stimulation and Inhibition of Plasminogen Activation (Fig. 1). TNF induced an early, brief increase in overall plasma PA activity. Plasma PA activity was already significantly increased in the first blood sample taken after TNF injection, i.e., after 15 min, and reached a maximum after 1 h (438 ± 13%; $p < 0.0001$ by ANOVA). Thereafter, plasma PA activity rapidly declined, returning to control values after 5 h. The increase in plasma activity coincided with rises in the plasma concentrations of u-PA antigen and t-PA antigen. u-PA antigen showed a modest increase from 3.4 ± 0.3 to 5.6 ± 0.4 ng/ml at 45 min ($p < 0.0001$ by ANOVA). Plasma t-PA antigen rose markedly from 4.0 ± 0.9 ng/ml to a maximum of 84.8 ± 15.9 ng/ml after 3 h ($p < 0.0001$ by ANOVA). The plasma levels of PAI-1 did not change in the first hour after TNF administration. Thereafter, a steep increase in PAI-1 antigen values was registered (occurring simultaneously with the decrease in plasma PA activity) with a peak after 3 h (from 4.9 ± 1.0 to 39.4 ± 7.8 ng/ml; $p < 0.0001$ by ANOVA). The plasma concentrations of u-PA-PAI-1 complexes did not change after TNF injection (data not shown). t-PA-PAI-1 complexes rose after TNF administration peaking after 2 h (from 0.07 ± 0.02 to 0.67 ± 0.13 nmol/liter, $p < 0.0001$ by ANOVA). Plasma plasminogen activity was not significantly affected by TNF injection (data not shown).

Plasmin Activity and Inhibition (Fig. 2). TNF administration elicited a steep increase in D-dimer plasma levels, reaching a summit after 1 h (from 81 ± 9 to 1,091 ± 257 $\mu g/ml$; $p < 0.0001$ by ANOVA). PAP complexes rose transiently after TNF injection, peaking after 45 min (from 3.1 ± 0.7 to 112.7 ± 25.2 nmol/liter, $p < 0.0001$ by ANOVA), while α_2-antiplasmin activity transiently decreased, reaching a nadir after 45 min (from 88 ± 3% to 73 ± 3%; $p < 0.01$ by ANOVA).

Discussion

The findings of this study are compatible with enhanced fibrinolytic activity mainly in the first 2 h after TNF injec-

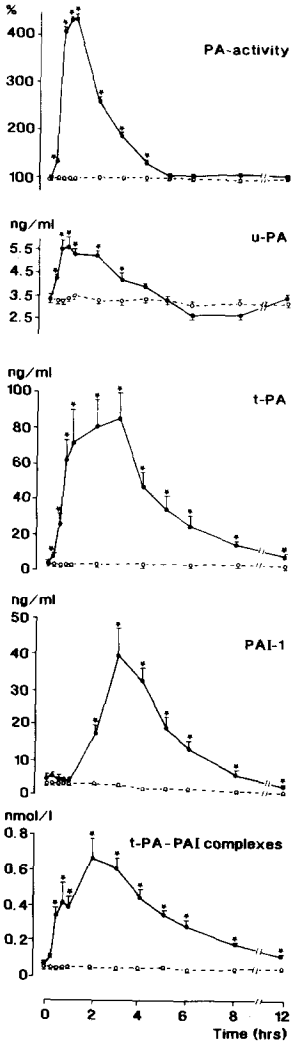

Figure 1. Stimulation and inhibition of plasminogen activation. Mean (± SEM) plasma levels of indexes of stimulation and inhibition of plasminogen activation after intravenous bolus injections of recombinant human TNF (50 $\mu g/m^2$; *filled circles*) or an equivalent volume of isotonic saline (*open circles*): PA activity, u-PA antigen, t-PA antigen, PAI-1 antigen, and t-PA-PAI-1 complexes. Asterisks indicate statistical significance for the comparison of TNF with saline ($p < 0.05$ by Newman-Keul's test for multiple comparison).

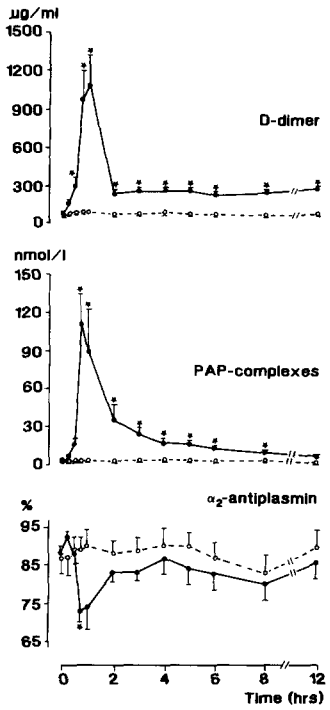

Figure 2. Plasmin activity and inhibition. Mean (± SEM) plasma levels of indexes of plasmin activity and inhibition after intravenous bolus injections of recombinant human TNF (50 $\mu g/m^2$, *filled circles*) or an equivalent volume of isotonic saline (*open circles*): D-dimer, PAP complexes, and α_2-antiplasmin activity. Asterisks indicate statistical significance for the comparison of TNF with saline ($p < 0.05$ by Newman's Keul's test for multiple comparison).

tion. Our study confirms and extends previous uncontrolled investigations in cancer patients infused with TNF. Silverman et al. (8) registered subsequent increases in plasma t-PA antigen and PAI activity, associated with a transient stimulation of plasma PA activity as determined in one patient. Van Hinsbergh et al. (9) measured several indexes of fibrinolysis at 3 and 24 h after the start of a continuous 24-h intravenous TNF infusion and found sustained elevations in t-PA antigen and PAI activity. In contrast to our study, u-PA antigen levels were not affected by TNF administration, which may have been related to the relative late blood sampling. The plasma levels of fibrin degradation products were elevated at the end of the TNF infusion. Remarkably, this increase was not significant after 3 h, which sharply contrasts with the early stimulation of fibrinolysis observed in our study (9). Factors that may have contributed to this apparent discrepancy include differences in the way of TNF administration (continuous infusion vs. bolus injection) and differences in the subjects under investigation (cancer patients vs. healthy humans). With respect to the latter possibility, it is noteworthy that patients with cancer often have subclinical activation of both the coagulation system and the fibrinolytic system (7–9).

The mechanisms underlying the in vivo effects of TNF on the fibrinolytic system are not elucidated by our study. In cultures of human endothelial cells, TNF decreases t-PA release (4), while high concentrations of TNF have been reported to increase t-PA secretion (6). u-PA and PAI-1 production are stimulated by TNF (4, 6, 17). However, all these TNF-induced changes in in vitro systems are detected only after several hours, and can therefore not properly explain the rapid in vivo fibrinolytic response observed in our study. Also, the possible involvement of secondary mediators induced by TNF remains speculative. IL-1 reduces t-PA release and stimulates u-PA and PAI-1 production by cultured endothelial cells (4, 17, 18), whereas thrombin increases both t-PA and PAI-1 secretion under the same experimental conditions (19). However, these effects, as for those provoked by TNF, occur relatively slowly. The rapidity by which the fibrinolytic system is activated after TNF injection as seen in this study is more suggestive for an effusion of stored plasminogen activators, probably from the vascular endothelium.

Interestingly, previous studies investigating the in vivo fibrinolytic response to intravenous endotoxin in healthy humans showed comparable results in our study after TNF injection, with the important difference that the TNF-induced changes were detected 1–2 h earlier (20, 21). Since this interval is identical to the time in which serum TNF reaches peak values after injection of endotoxin (21), these combined data strongly implicate TNF as an intermediate factor in the fibrinolytic changes in endotoxemia and septicemia.

We have recently reported the time course of the activation of the coagulation system after injection of TNF in the same volunteers (10). TNF induced a rapid activation of factor X, peaking after 45 min, followed by a gradual generation of thrombin as reflected by elevated plasma concentrations of the prothrombin activation peptide F1+2, becoming apparent after 2 h and persisting for 6–12 h (10). This slow and sustained thrombin formation contrasts with the presently described fibrinolytic response characterized by profound activation in the first hour postinjection, followed by a fast inhibition. Direct comparison of the dynamics of procoagulant and fibrinolytic effects of TNF in our study subjects reveals that fibrinolysis was stimulated before significant thrombin generation could be detected in plasma, which, however, does not exclude thrombogenesis at the microvascular-endothelial level as an initiating event in the observed fibrinolytic response. Still, our findings challenge the putative role of fibrinolysis as a reactive process protecting against intravascular coagulation. Moreover, when prothrombin activation became maximal, fibrinolysis was already offset. The highly different time courses of the fibrinolytic and coagulative responses to intravenous TNF led us to hypothesize that a net procoagulant state may exist several hours after TNF injection. This remarkable disbalance in the procoagulant and fibrinolytic mechanism may be of importance for our understanding of the tendency towards microvascular thrombosis in septicemia.

In conclusion, this controlled study demonstrates that intravenous injection of TNF induces a rapid activation and a subsequent inhibition of the fibrinolytic system in healthy humans. Our results, taken together with the data obtained in the coagulation study (10), suggest that excessive release of TNF into the circulation, precipitated by invading bacteria early in the course of septicemia, may play an important part in the ensuing development of a disbalance between coagulant and fibrinolytic mechanisms, eventually resulting in microvascular thrombosis.

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